



Effect of Nano-selenium on Plasma Antioxidant status and Reproductive system function of Female Rats exposed to Oxidative Stress Induced by Doxorubicin

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ABSTRACT: The present study was designed to examine the protective effect of nano-Selenium (nSe) on plasma antioxidant capacity and the concentration of gonadotropic and steroidal hormones in female rats following exposure to doxorubicin. Rats were randomly divided into four experimental groups (5 rats in each) and treated as follows: group 1 received normal saline by injection (ip) daily, group 2 received doxorubicin (6 mg/kg body weight) dissolved in normal saline, group 3 received nSe (0.3 mg/kg body weight) by ip injection, and group 4 received doxorubicin (6 mg/kg body weight) dissolved in normal saline following nSe (0.3 mg/kg body weight) injected one day before. All groups were treated for 28 days and injections were done each 48 hour. Administration of doxorubicin significantly increased superoxide dismutase activity as compared with control group and supplementation of nSe had no effect on it. Rats in control group had the lowest malondialdehyde level and those received doxorubicin had the highest level. Rats received combination of doxorubicin and nSe had lower malondialdehyde level compared to those in doxorubicin alone. There were no significant differences among treatments for Plasma FSH and estrogen concentrations. The highest concentration of LH was found for rats received combination of doxorubicin and nSe and the lowest one was for those received doxorubicin alone. The highest concentration was for rats received combination of doxorubicin and nSe and the lowest one was related to rats in group received doxorubicin alone. Findings of the present research help us to conclude that the nSe in dosages used in this study, has improving effects on plasma progesterone concentration in female Wistar rats exposed to doxorubicin.

Keywords: Doxorubicin, Estrogen, Nano-Selenium, Progesterone, Rat

INTRODUCTION

The doxorubicin is an anticancer drug which used in treatment of various tumors. This drug exhibits severe toxicity to the reproductive system and resulted in disturb the male and female fertility (Zanetti *et al.*, 2007; Sridevi, 2011; Sridevi *et al.*, 2012). A number of possible toxic mechanisms have been recognized following exposure to doxorubicin, the main pathogenic mechanism appears to involve the generation of reactive oxygen species named oxidative stress (Hozayen, 2012; Patil and Balaraman, 2009; Abdel-Wahab *et al.*, 2003). Oxidative stress occurs as a result of an imbalance between pro-oxidants and antioxidants (Al-Gubory *et al.*, 2010). This imbalance is due to increased levels of reactive oxygen species, nitrogen species or decreased antioxidant defense system occurs (Burton and Jauniaux, 2010; Cindrova-Davies *et al.*, 2007; Ruder *et al.*, 2009).

If the production of reactive oxygen species be more than usual, it can damage the cells, including damage to DNA, lipid membranes, and proteins. Oxidative oxidants and its control by antioxidants is one of the important topics in animals' physiology of the female reproductive system. The overall reactive oxygen species have an important transitional role in the regulation of ovulation, oocyte maturation, corpus luteum formation, uterine activity, fetal cycle, embryo implantation and development of the placenta and the fetus through diverse signaling and transition pathway, but the imbalance between the production of reactive oxygen species and antioxidants is a reason for the start and spread of damage to the reproductive process. Oxidative stress is one of the factors that cause infertility or recurrent miscarriages, endometriosis, polycystic ovarian syndrome and other disorders related to pregnancy (Webster *et al.*, 2008).

On the other hand, the ability of selenium (Se) to delay oxidative processes have been documented extensively (Cai *et al.*, 2012). Selenium is known to be an antioxidant as it is able to decrease the Malondialdehyde concentrations near normal concentration (Placha *et al.*, 2014; Boostani *et al.*, 2015). The essential functions of Se in mammals are mediated by a group of 25 selenoproteins in which Se is in the form of selenocysteine, the Se-containing homolog of cysteine. Enzymatic activities have been assigned to 12 of these selenoproteins; four are forms of glutathione peroxidase. With the recent development of nanotechnology, nano-selenium (nSe) has attracted widespread attention. Because nano-particle exhibits novel properties, such as great specific surface area, high surface activity, a lot of surface active centers and high catalytic efficiency, high absorbed ability and low toxicity (Wang *et al.*, 2007; Zhang *et al.*, 2008). Further studies are needed to find the mechanism of the nano material defensive effects. Therefore, the present study was designed to examine the protective effect of nSe on plasma antioxidant capacity and the concentration of gonadotropic and steroidal hormones in female rats following exposure to doxorubicin.

MATERIALS AND METHODS

Doxorubicin from were purchased Korea United Pharfma (Seoul, South Korea) and nSe from American Elements Company (Los Angeles, USA). The particle size of nano-selenium ranged from 20 to 60 nm with 99.95% purity. The nSe particles were suspended in 1% sodium carboxymethyl cellulose as stabilizer, stirred with magnetic stirrer for 15 minutes and then dispersed by ultrasonic vibration for 20 min. In order to avoid the aggregation of the particles fresh suspension was prepared before every use. Twenty newly weaned female Wistar albino rats (45-55 g body weight) were obtained from the Razi Institute (Karaj, Iran). The animals were housed in plastic cages, fed a standard laboratory diet and water ad libitum. They were kept under standard conditions of temperature (23 ± 2 °C), and 12h light/dark period. Animal handling and care were performed in accordance with the guidelines established by the Razi institute guidelines on Animal Care. The animals were quarantined for 10 days before beginning the experiments. After one week of acclimatization to the laboratory conditions, rats were randomly divided into four experimental groups (5 rats in each) as follows: In this study, four groups each containing five female rats were used. Treatment groups were as follows: group 1 received normal saline by injection (ip) daily, group 2 received doxorubicin (6 mg/kg body weight)dissolved in normal saline, group 3

received nSe(0.3 mg/kg body weight) by ip injection, and group 4 received doxorubicin (6 mg/kg body weight) dissolved in normal saline following nSe(0.3 mg/kg body weight) injected one day before. All groups were treated for 28 days and injections were done each 48 hour.

After 28 days, rats were fasted overnight with free access to water. Rats were anesthetized with diethyl ether and blood was collected into heparinized tubes from heart. The blood was then centrifuged and the plasma was collected and kept at -80 °C for the determination of luteinizing hormone (LH), follicle-stimulating hormone (FSH), estrogen and progesterone. Hormones of LH, FSH and estrogen were measured using enzyme-linked immunosorbent assay (ELISA) kits. Briefly, this assay employs the competitive inhibition enzyme immunoassay technique. The micro titer plate provided in these kits had been pre-coated with goat-anti-rabbit antibody. Standards or samples were added to the appropriate micro titer plate wells with an antibody specific for hormone and Horseradish Peroxidase conjugated hormone. The competitive inhibition reaction was launched between with HRP labeled hormone and unlabeled hormone with the antibody. A substrate solution was added to the wells and the color develops in opposite to the amount of hormone in the sample. The color development was stopped and the intensity of the color measured.

The progesterone ELISA Kit for rat is based on the principle of competitive binding. An unknown amount of progesterone present in the sample and a defined amount of progesterone conjugated to horseradish peroxidase compete for the binding sites of progesterone antiserum coated to the wells of a micro plate. After incubation on a shaker the micro plate was washed four times. After addition of the substrate solution, the concentration of progesterone was inversely proportional to the optical density measured.

Plasma levels of malondialdehyde as a product of lipid peroxidation was measured by the thiobarbituric acid assay. Absorbed by spectrophotometrically method (Shimadzu UV-260, Shimadzu Corp, Tokyo, Japan) in 523nm. The contents of malondialdehyde were expressed as $\mu\text{mol/mg-protein}$ (Ohkawa *et al.*, 1979). An indirect method of inhibiting auto-oxidation of epinephrine to its adrenochrome was used to assay superoxide dismutase activity in the plasma.

Data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS software. Mean comparison was done using the Duncan's Multiple Range Test at $P<0.05$.

RESULTS AND DISCUSSION

The main aim of present study was to evaluate the effect of doxorubicin on plasma antioxidant capacity and the concentration of gonadotropic and steroidal hormones and the other purpose was to evaluate the protective effect of nSe on them. In the literature, there is no report on the effects of these factors on plasma antioxidant capacity and the gonadotropic and steroidal hormones.

The effect of doxorubicin and nSe on the superoxide dismutase activity is shown in Figure 1. Injection of doxorubicin significantly increased superoxide dismutase activity as compared with control group and supplementation of nSe numerically decreased it compared with those received doxorubicin alone. Oxidative stress induced by doxorubicin is cause of increase in superoxide dismutase enzyme activity.

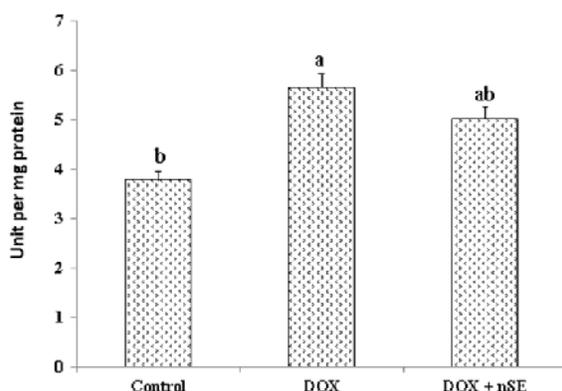


Fig. 1. Effect of doxorubicin and nSe administration on plasma superoxide dismutase concentration

Fig. 2 shows plasma Malondialdehyde levels of rats in different treatments. Rats in control group had the lowest malondialdehyde level and those received doxorubicin had the highest level. Rats received combination of doxorubicin and nSe had lower malondialdehyde level compared to those in doxorubicin alone. Doxorubicin produces persistent damage to the reproductive organ cells (Saalu *et al.*, 2010), as well as increase in their oxidative stress. Although a number of possible toxic mechanisms have been recognized following exposure to doxorubicin, the main pathogenic mechanism appears to involve the generation of reactive oxygen species (Hozayen, 2012; Patil and Balaraman, 2009). Some experimental studies have shown that reactive oxygen species are important agents for tissue damage (Malekirad *et al.*, 2011).

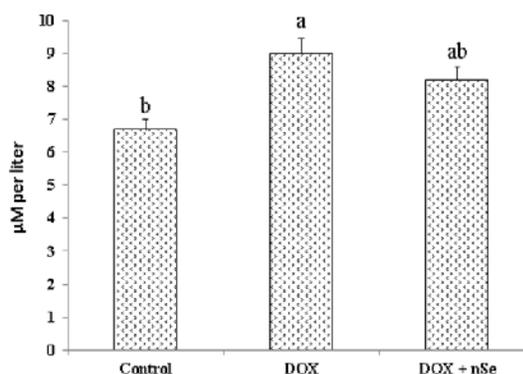


Fig. 2. Effect of doxorubicin and nSe on plasma malondialdehyde concentration.

It has been demonstrated that oxygen radical-induced damage of lipids in membrane is the key factor for doxorubicin-induced toxicity (Abdel-Wahab *et al.*, 2003). These processes also occur in the testis of doxorubicin-treated animals, elucidating the high susceptibility of proliferating germ cells and directing to pretreatment with antioxidants as a promising form of reducing doxorubicin toxicity (Zanetti *et al.*, 2007). The finding of this study is consistent with findings of Rotruck *et al.* (1973). They reported that Selenium is an important and effective part in activities of antioxidant enzyme glutathione peroxidase, which is set up to control the levels of hydrogen peroxide and lipid peroxide during normal metabolic activity and also stress.

The effect of doxorubicin and nSe administration on plasma FSH concentration is presented in Figure 3. Based on Duncan test, there were no significant differences among treatments for Plasma FSH concentration. Numerically, the lowest concentration was for rats received doxorubicin and the highest concentration was for rats received combination of doxorubicin and nSe. The effect of doxorubicin and nSe administration on plasma estrogen concentration is presented in Figure 4. Based on Duncan test, there were no significant differences among treatments for plasma progesterone concentration. The effect of doxorubicin and nSe administration on plasma LH concentration is presented in Fig. 5. There were significant differences among treatments for plasma concentration of LH. The highest concentration of LH was found for rats received combination of doxorubicin and nSe and the lowest one was for those received doxorubicin alone. The cause of the reduction in plasma LH and FSH levels of rats received doxorubicin maybe high plasma corticosterone levels (Artykova *et al.*, 1977) as animals subjected to oxidative stress.

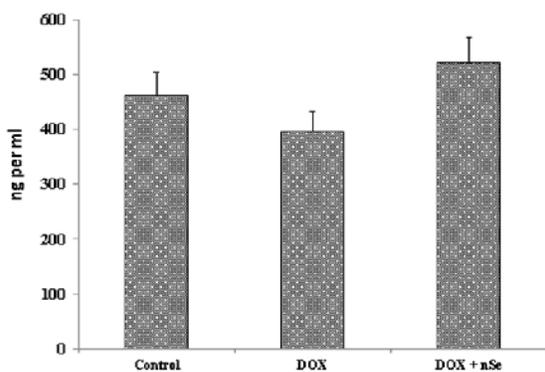


Fig. 3. Effect of doxorubicin and nSe administration on plasma FSH concentration.

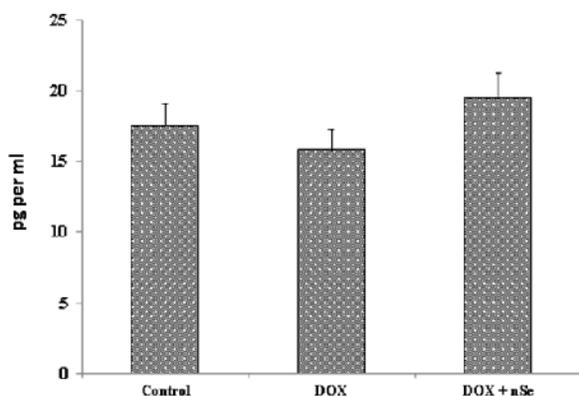


Fig. 4. Effect of doxorubicin and n Se administration on plasma estrogen concentration.

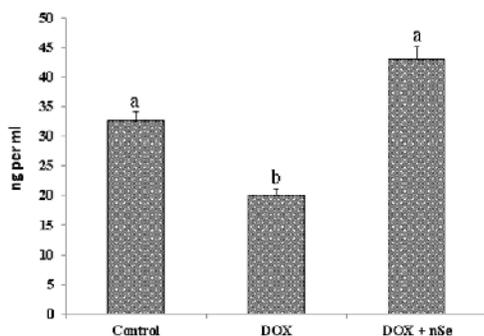


Fig. 5. Effect of doxorubicin and nSe administration on plasma LH concentration.

High corticosterone can reduce plasma gonadotropin and steroids levels (Hardy *et al.*, 2005; Vreeburg *et al.*, 1988). The effect of doxorubicin and nSe administration on plasma progesterone concentration is presented in Fig. 6. There were significant differences among treatments for progesterone concentration of plasma. The highest concentration was for rats received

combination of doxorubicin and nSe and the lowest one was related to rats in group received doxorubicin alone.

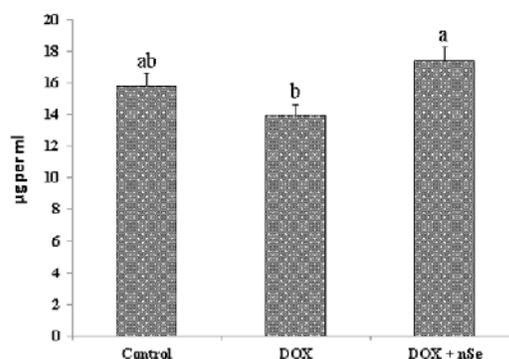


Fig. 6. Effect of doxorubicin and nSe administration on plasma progesterone concentration.

Consistent to our finding, in the study by Gökçe *et al.* (2011), oxidative stress reduced sex hormones levels that this reaction was significant only about progesterone. It was reported (Muthuvel *et al.*, 2006) that oxidative stress reduced LH and FSH. Oxidative stress can modulate cellular functions, and OS can impair the intracellular milieu resulting in diseased cells or endangered cell survival. In a study (Yang *et al.*, 2014), mice exposed to an oxidative stress inducer and found that inducer could induce DNA damage in endometrial cells and finally embryo loss. Recently, oxidative stress has been reported to have an important role in the normal functioning of the female reproductive system and in the pathogenesis of female infertility (Bedaiwy *et al.*, 2002; Agarwal and Allamaneni, 2004). Findings of the present research help us to conclude that the nSe in dosages used in this study, has improving effects on plasma progesterone concentration in female Wistar rats exposed to doxorubicin.

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